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A Review and Evaluation of Human Genetic Bioassay Data for Some Known, Probable, and Unclassified Human Carcinogens

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Research Triangle Park, NC

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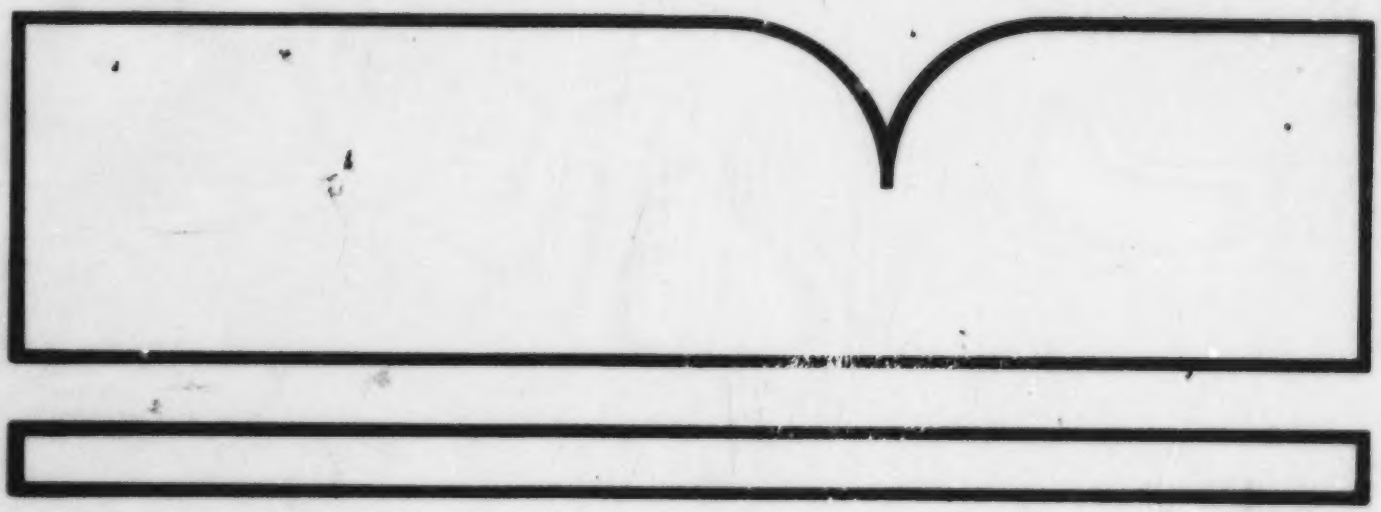

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16. ABSTRACT

The purpose of this paper is to review and to evaluate a subset of data base described by Waters et al. This data subset deals specifically with the application of genetic bioassys utilizing human cells, tissues, and body fluids.

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A REVIEW AND EVALUATION OF HUMAN GENETIC BIOASSAY DATA FOR SOME KNOWN, PROBABLE, AND UNCLASSIFIED HUMAN CARCINOGENS

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INTRODUCTION

The purpose of this paper is to review and to evaluate a subset of the data base described by Waters et al.[1] This data subset deals specifically with the application of genetic bioassays utilizing human cells, tissues, and body fluids.

METHODS

The procedure for collecting published papers has been described.[1] A selection of bioassay systems was made from the approximately 100 bioassays represented in the data base. The bioassays selected were those that 1) utilize human cells or tissues or 2) have been directly applied to body fluids or tissues from exposed human beings. The agents evaluated in either of the two bioassay system types were then selected by computer search from the larger data base. Papers dealing with combined chemical exposures were excluded as were reports concerning chemically induced sperm count depression or altered sperm morphology.

RESULTS

The results of the computer-assisted selection may be seen in Table 1. A matrix was printed by the computer which consisted of 22 compounds for which there were qualitative data in 1 or more of 17 different bioassay systems. The bioassay data retrieved are presented in this section according to bioassay group. The five bioassay groups are those which:

1) provide a measure of primary damage to the DNA of human cells by either a) monitoring the non-S phase incorporation of radiolabeled [^{3}H]-thymidine into DNA, so-called unscheduled DNA synthesis (UDS), or b) determining the extent of DNA strand breakage in human cells

2) measure the potential of an agent to inhibit the normal replicative synthesis of DNA in human cells in culture

3) determine the frequency of sister-chromatid exchange (SCE) formation in human cells exposed in vitro or in vivo

4) examine and quantitate gross chromosomal aberrations in human cells under in vitro or in vivo conditions

5) assess exposure to a genotoxic agent by use of an indicator organism (such as S. typhimurium) to detect mutagens in human body fluids or tissues.

Bioassays Measuring Primary Damage to Human Cell DNA (Bioassay Group 1)

Whiting et al.[2] have performed a comprehensive study on the effects of chromium salts on UDS and on DNA fragmentation as measured by alkaline sucrose gradient sedimentation. Hexavalent chromium, but not a chromic glycine complex, caused significant cytotoxicity, DNA damage, and DNA repair in cultured human cells. The specificity of chromium (VI) for SCE and aberrations was also reported by the same research group in a companion paper.[3] The ranges of concentration of chromate in which effects were detected in human skin fibroblasts are shown in Table 2. The lower limit for detection of genetic damage in the various assays spanned four orders of magnitude. Increases in SCE's were observed at low concentrations without apparent toxicity, but maximum frequencies of SCE and gross aberrations were limited by the toxic effects of chromate. DNA repair and fragmentation were observed at concentrations and time for which colony formation from single cells were inhibited. The techniques used with the various assays differed to some extent, but the concentraions shown in Table 2

Table 1 (computer printout)

3 to 6 pages

Table 2. Ranges of Concentration of Chromate in Which Effects Were Detected in Cultured Human Skin Fibroblasts[a]

Effect	Concentration Limits (M) for Detection of Effect	
	Lower	Upper
Sister chromatic exchanges[b]	$3 \cdot 10^{-8}$	10^{-6}
Inhibition of colony formation		
chronic exposure	10^{-7}	-
acute exposure	$5 \cdot 10^{-6}$	-
Chromosomal aberrations[b]	$8 \cdot 10^{-7}$	$2 \cdot 10^{-6}$
DNA repair	10^{-6}	$3 \cdot 10^{-3}$
DNA fragmentation	$5 \cdot 10^{-4}$	-

[a]Taken from Whiting et al.[2]
[b]Data from MacRae et al.[3]

were thought to provide a reasonable estimate of the relative sensitivities of the assays to chromate.

Unscheduled DNA synthesis in human cells in culture (Bioassay Group 1a). HeLa cells. Among 51 compounds that Martin et al.[4] tested for induction of UDS in HeLa cells, the following results were obtained for 4 agents included in the data base. Aflatoxin B1 was active over the dose range 10^{-8} to 10^{-4} M in the presence of phenobarbitone-induced S-9. Exposure was carried out for 2.5 h. Benzidine, cyclophosphamide, and diethylstilbestrol (DES) were active under the same conditions of metabolic activation--the former compounds over the ranges of 10^{-7} to 10^{-3} M and 10^{-7} to 10^{-4} M, respectively, and the latter compound at the single concentration tested of 10^{-6} M. Insufficient data were presented to permit definitive comparison of the relative biological activity of the four compounds in the system. However, the relative activities of Aflatoxin B1 and benzidine observed in the UDS assay clearly do not reflect the substantial differences in activity observed in the Ames test or in rodent carcinogenesis bioassays that were reported by Meselson and Russell[5] (Fig. 1).

Human skin fibroblasts. Among 64 compounds, San and Stich[6] examined the effect of Aflatoxin B1 on the induction of UDS in diploid human skin fibroblasts. They observed [^{3}H]-thymidine uptake between approximately 10^{-5} and 5×10^{-4} M after a 30 min exposure to the compound in the presence of exogenous metabolic activation. Stich and Laishes[7] examined the possible specificity of exogenous metabolic activation systems from liver, lung, and kidneys of various animals for aflatoxin B1; they found no

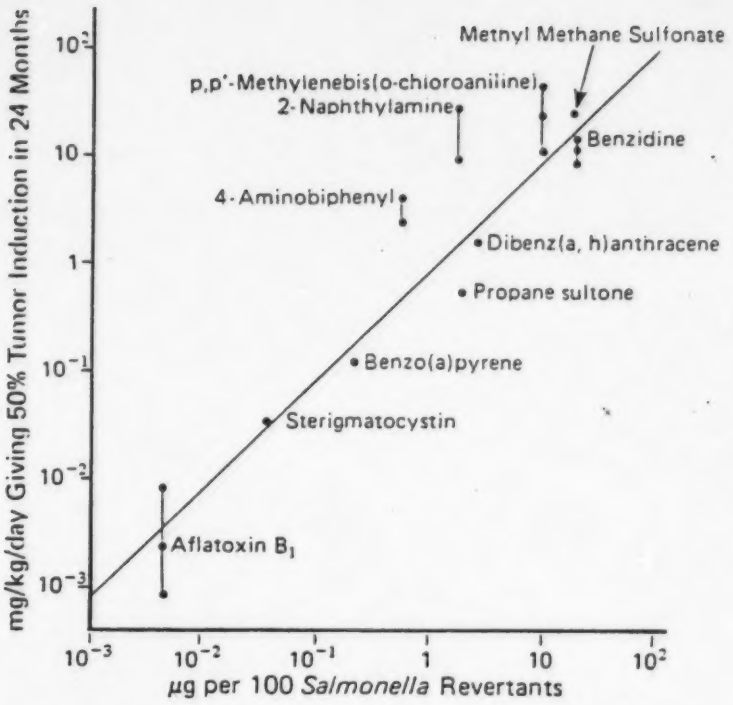

Figure 1. Relation between mutagenic and carcinogenic potency. Used with permission of Meselson and Russell.[5]

significant difference, although the carcinogenic effect of aflatoxin B1 differs greatly for the species used. In the same paper, Stich and Laishes compared the relative sensitivity of normal and Xeroderma pigmentosum (XP-E) fibroblasts with respect to the induction of UDS after exposure to aflatoxin B1 (2×10^{-4} M), and they found about 20 to 25% less UDS in the XP-E fibroblasts. However, the XP-E cells were more sensitive than normals to chromosome-damaging and lethan effects of aflatoxin B1.

Human bone marrow cells. The Swedish team of Lewensohn and Ringborg[8] examined the effect of the bifunctional alkylating agent melphalan on UDS in human bone marrow cells and in peripheral leukocytes. After a 30 min exposure to 10^{-6} to 10^{-3} M melphalan, UDS increased in the absence of an exogenous activation system. The various types of bone marrow cells were examined individually for their UDS levels, and levels were generally greatest in the blast cells, i.e., myeloblasts (and promyelocytes) and erythroblasts.

DNA strand breaks in human skin fibroblasts (Bioassay Group 1b). Nordenskjold et al.[9] have described a procedure for studying DNA strand breaks using alkaline denaturation followed by hydroxylapatite column chromatography. The bifunctional alkylating agents cyclophosphamide and melphalan were tested in the system. In agreement with previous information, melphalan was direct acting, while cyclophosphamide required rat liver S-9 metabolic activation to induce DNA strand breakage in the normal human fibroblasts. Fig. 2 shows the time-related and concentration-related DNA strand breaks after exposure to melphalan at 10^{-5}, 10^{-4}, and 10^{-3} M. After removal of the drug at 0.5 h, DNA strand breaks decreased to background levels during a 21 h period, indicating DNA repair. The results for cyclophosphamide at 5×10^{-5}, 5×10^{-4}, and 5×10^{-3} M are shown in Fig. 3. In contrast with the results for melphalan, the DNA strand breakage level did not decrease within 21 h after removal of cyclophosphamide at 60 min. According to the authors, the initial decrease in single-stranded DNA eluted suggests the presence of interstrand crosslinks. This follows, since after a certain amount of chain breakage has occurred, crosslinks will result in an increase in the DNA fraction eluted as double stranded from hydroxylapatite. Thus, an apparently lower rate of DNA strand breaks will be recorded.

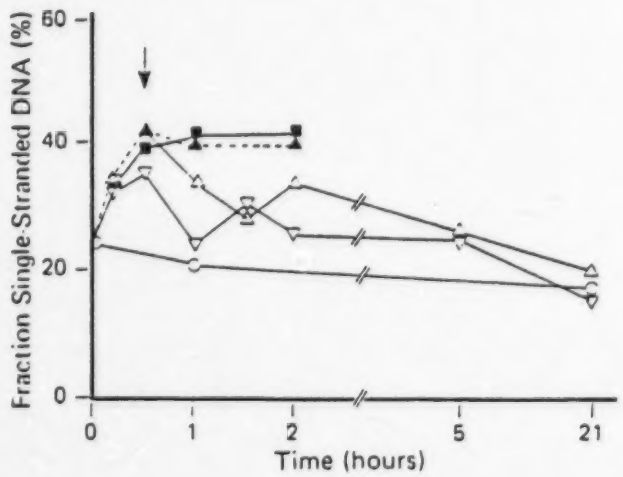

Fig. 2. Induction of DNA strand breaks in human fibroblasts by melphalan. Cells were incubated at 37°C in complete medium without melphalan (o) or with melphalan at 1×10^{-5} M (▽), 1×10^{-4} M (△), and 1×10^{-3} M (■) concentrations. Open symbols indicate incubations from which the drug was omitted after 30 min of incubation (arrow). Solid symbols indicate incubations in which the drug was present throughout the incubation. Used with permission of Nordenskjold et al.[9]

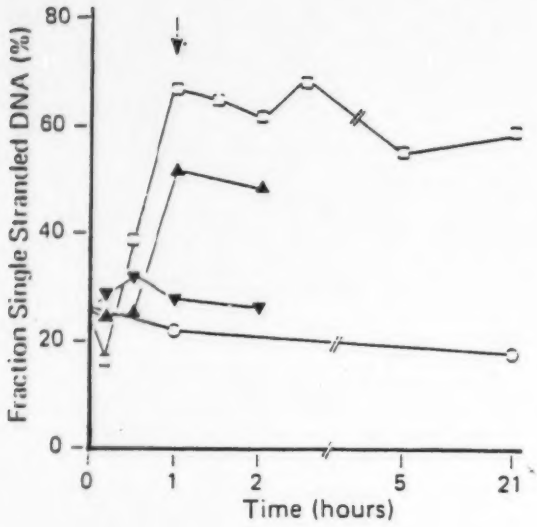

Fig. 3. Induction of DNA strand breaks in human fibroblasts by cyclophosphamide at O (o), 5 x 10^{-5} M (∇), 5 x 10^{-4} M (Δ), and 5 x 10^{-3} M ( ) concentrations. Cells were incubated with liver microsomes (0.7 mg protein/ml) from phenobarbital-treated rats, an NADPH-generating system, and cyclophosphamide. Open symbols indicate incubations from which the drug was omitted after 60 min of incubation (arrow). Solid symbols indicate incubation in which the drug was present throughout the incubation. Used with permission of Nordenskjold et al.[9]

Inhibition of DNA Synthesis (Bioassay Group 2)

Inhibition of DNA synthesis in HeLa cells. In 1978, Painter[10] described a model which measures inhibition of incorporation of [^{3}H]-thymidine. After controls and treated cultures are prelabeled with ^{14}C-thymidine, ^{3}H/^{14}C ratios of treated cells are divided by the ^{3}H/^{14}C ratios of controls, and the data are plotted as percentages of control incorporation into DNA. Aflatoxin B1, benzidine, and cyclophosphamide have been evaluated in this system, and the results have been compared with those of the Ames test.[11] The concentration that inhibited HeLa cell DNA synthesis by 40% within 2.5 h after removal of the agent from the cells, i.e., the "effective molarity," was plotted against the yield of mutants (in units of revertants per nanomole) in the Salmonella system. The results obtained are illustrated in Fig. 4. Aflatoxin B1 was effective at 5 x 10^{-8} M in the presence of S-9. Benzidine was directly active at an effective molarity of 6 x 10^{-4} M, and cyclophosphamide was effective at 6 x 10^{-4} M in the presence of S-9.[10] These relative activity values compare favorably with those determined for the same compounds in the Ames test.[11]

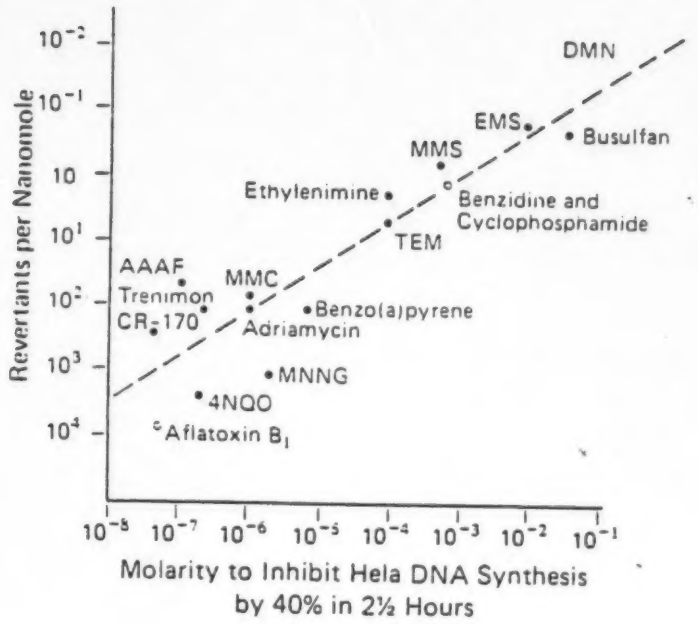

Fig. 4. A comparison of the relative effectiveness of various chemicals to inhibit HeLa DNA synthesis and to form revertants of mutants of Salmonella typhimurium: •, Agents that did not require metabolic activation with S-9; o, agents that did require activation with S-9. The dashed line represents the best fit for the 17 points on the graph but does not imply statistical significance. Abbreviations: DMN, dimethylnitrosamine; EMS, ethyl methanesulfonate; MMS, methyl methanesulfonate; TEM, triethylenemelamine; MMC, mitomycin C; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; AAAF, N-acetoxy-2-acetylaminofluorene; 4NQO, 4-nitroquinoline-1-oxide. Used with permission of Painter and Howard.[11]

Sister-Chromatid Exchange in Human Cells (Bioassay Group 3)

Data from SCE studies are presented below in two categories: in vitro studies and in vivo exposure. Also, because in most cases SCE studies were performed using human lymphocytes, these results are organized alphabetically by compound.

In vitro SCE. Aflatoxin B1. Elevated SCE frequencies were observed in human peripheral lymphocytes from healthy donors in an Egyptian study[12] at 72 h following exposure to Aflatoxin B1 in vitro at concentrations of 0.01 and 0.05 μg/ml (3.2×10^{-5} and 1.6×10^{-4} M) in the medium. Nonrandom chromosome breaks and

interchanges were also reported. The results of this study imply that human lymphocytes possess some ability to metabolically activate aflatoxin.

Arsenic. Zanzoni and Jung[13] of West Germany reported that inorganic trivalent arsenic (Na_2HAsO_4), 10^{-6} M and 2×10^{-6} M, present for 72 h in cultures of normal human lymphocytes, induced a dose dependent increase in SCE per cell, which was significantly different from untreated controls at the higher concentration. Cell survival remained high at these concentrations. Chromosome aberrations recorded as breaks and pulverized chromosomes were observed at concentrations of 2×10^{-6} M to 2×10^{-5} M, which coincided with reduced cell survival.

Benzene. According to a 1978 paper by Gerner-Smidt and Friedrich,[14] benzene failed to change the SCE rate or number of chromosomal aberrations, compared to controls, when presented to human lymphocytes in vitro at 1.52 µg/ml or 1.52 mg/ml (1.9×10^{-5} M or 1.9×10^{-2} M) for 72 h in the absence of an exogenous metabolic activation system. Benzene did not inhibit cell growth at these concentrations.

An Argentinian group reported[15] in abstract form in 1979 that human lymphocyte cultures exposed to benzene in the presence of rat liver microsomes for 1 h or in the absence of exogenous activation for the first 24 h of culture exhibited elevated SCE/cell observed at 72 h. Exposure to benzene without exogenous activation for the last 48 or 18 h of culture inhibited SCE. Exposure concentrations were not provided.

Morimoto and Wolff[16] reported the results of a study on benzene and three of its metabolites--phenol, catechol, and hydroquinone--in human lymphocyte cultures. The study examined SCE and cell cycle kinetics. Shown in Table 3 are the estimated concentrations of benzene and its metabolites required to induce cytogenetic and cytotoxic effects. Exposure was for 72 h in the absence of exogenous metabolic activation. Benzene did not increase SCE nor delay cell turnover, in contrast with its metabolites. Catechol was the most toxic of the metabolites, and phenol was the least toxic. The SCE frequency in cells exposed to benzene, phenol, hydroquinone, or catechol as a function of exposure concentration is shown in Fig. 5. Elevated SCE frequencies were observed with benzene metabolites only at concentrations sufficient to cause significant (50% or greater) reduction in the mitotic index.

Table 3. Estimated Concentration (M) of Benzene and its Metabolites Required to Induce Cytogenetic and Cytotoxic Effects[a]

Effect	Catechol	Hydro-quinone	Phenol	Benzene
SCE doubling	3×10^{-5}	9×10^{-5}		
12 h delay of cell cycle	2×10^{-5}	1×10^{-4}	5×10^{-4}	
50% reduction in mitotic index	3×10^{-5}	4×10^{-5}	7×10^{-4}	8×10^{-4}

[a]Taken from Morimoto and Wolff.[16]

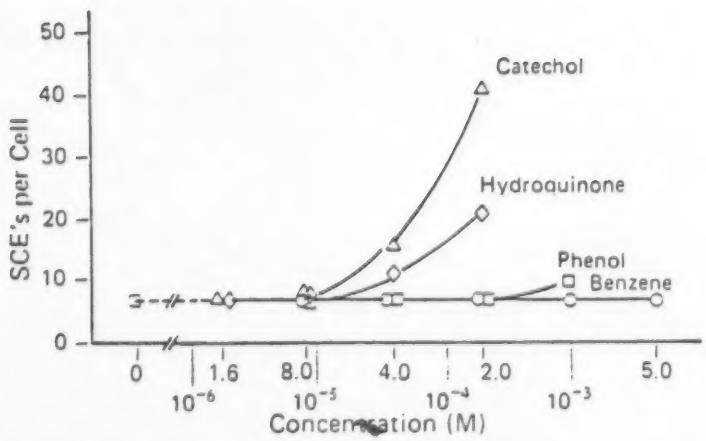

Fig. 5. The SCE frequency in cells exposed to benzene or one of its metabolites. The standard error of the mean is not shown, because it is less than 6% of the mean at each point. Used with permission of Morimoto and Wolff.[16]

Diethylstilbestrol. A study was reported by Rudiger and colleagues[1/] in West Germany on SCE induction in human skin fibroblasts by diethylstilbestrol (DES) and two of the DES metabolites, DES-α,β-oxide and β-dienestrol (Fig. 6). The former metabolite results from oxidation of the olefinic α,β-double bond and is an electrophilically active epoxide. The latter metabolite is formed by peroxidases, probably via highly reactive semiquinones and quinones. It can be further oxidized to ω-hydroxy-β-dienestrol, which can form reactive esters. A third metabolic pathway involves orthohydroxylation relative to the phenolic hydroxyl groups, leading to highly reactive catechols. With the two metabolites tested, DES-α,β-oxide and dienestrol, SCE frequency was increased at lower concentrations than with DES (Fig. 7), suggesting that the induction of SCE by DES required metabolic activation. In the presence of α-naphthoflavone, a monooxygenase

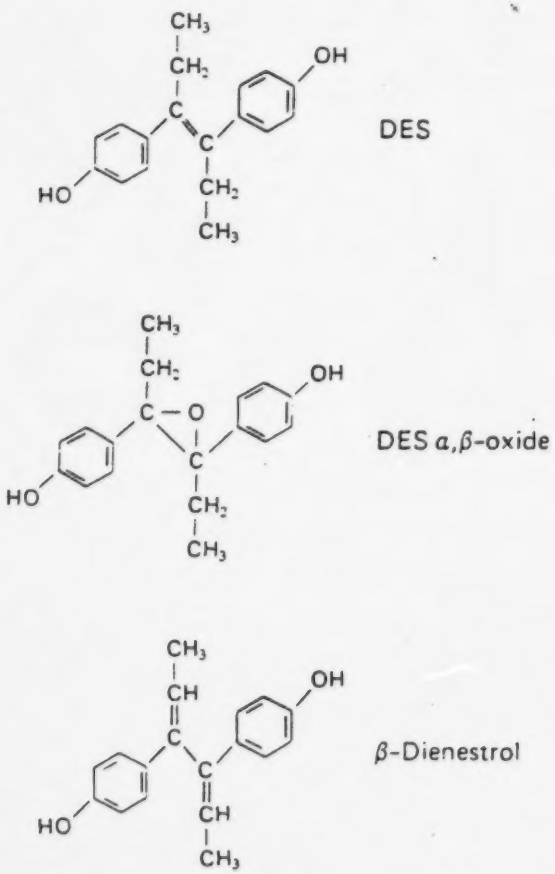

Fig. 6. Chemical structure of diethylstilbestrol (DES), and the two metabolites used for SCE induction. Used with permission of Rudiger et al.[1/]

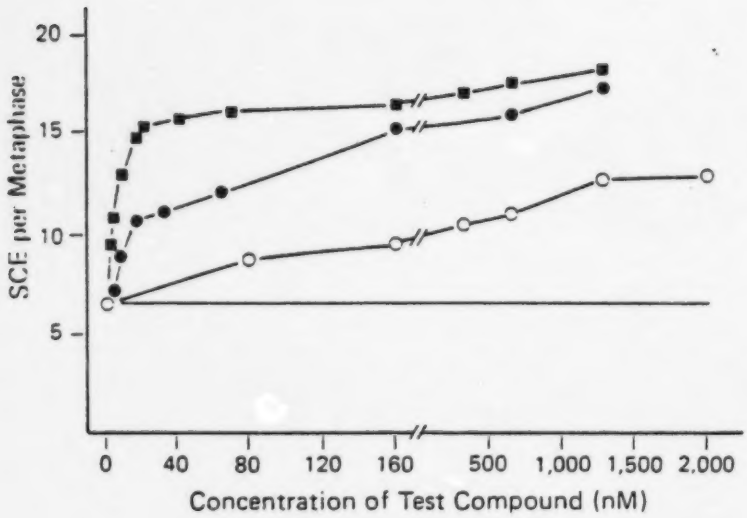

Figure 7. SCE induction by DES (o) DES-α,β-oxide, (o) and β-dienestrol (■). Used with permission of Rudiger et al.[17]

inhibitor, the induction of SCE by DES and β-dienestrol were markedly inhibited, suggesting that both these compounds require metabolic activation by monooxygenase(s) for induction of SCE.

Epichlorohydrin. Epichlorohydrin was evaluated for its ability to induce SCE in human lymphocytes in vitro in a study by White.[18] The compound induced SCE in a concentration-dependent fashion after a 2 h exposure at 2×10^{-4} M (twofold increase over control) and at 4×10^{-4} M (threefold increase over control). As would be expected for a direct-acting mutagen, the presence of rat liver S-9 reduced the apparent activity: The concentrations required to produce a threefold increase in SCE yield over control was raised from 4×10^{-4} M without activation to 10^{-3} M with activation (Fig. 8).

Ethylene oxide. The effects of ethylene oxide on SCE in human skin fibroblasts cultures were studied by Star.[19] Concentrations of 360 ppm in the liquid medium caused total cell destruction, but at 36 ppm the cells remained alive, and SCE frequency rose from 12.53 to 16. Endotracheal tubes containing residual ethylene oxide at 200 to 600 ppm also caused elevated SCE.

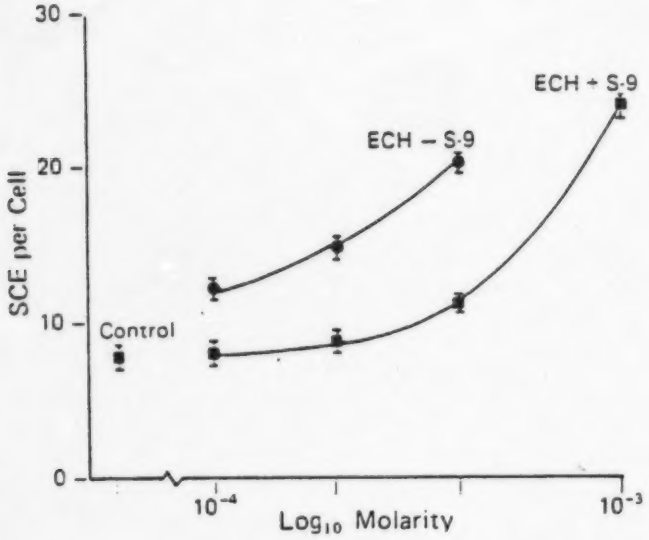

Fig. 8. The yield of SCE per cell induced in lymphocytes after exposure to epichlorohydrin for 2 h with and without metabolic activation. Used with permission of White.[18]

In vivo SCE. Arsenic. Burgdorf et al.[20] in 1977 described an elevated SCE frequency in lymphocytes of six patients treated with trivalent arsenic (as Fowler's solution, 1% $KAsO_2$ in H_2O). Administered arsenic for a variety of reasons (Table 4), all patients had stigmata (keratosis or diffuse pigmentary changes) of arsenic use as well as biopsy-proven skin cancers. The 6 arsenic-exposed patients had a mean of 14.00 (SD 3.12) SCE/mitosis, whereas 44 normal controls had a mean of 5.8 (SD 2.27) SCE/mitosis. There was no difference in chromosomal breakage between the two groups. The characteristics of the six patients exposed to arsenic are shown in Table 4; noteworthy is the latency period that was generally >25 years. The authors conclude that arsenic is implicated in the etiology of multiple cutaneous malignancies in the six patients and suggest that the significantly elevated SCE in cultured lymphocytes from these patients may be related to arsenical carcinogenesis.

Ethylene oxide. Twelve hospital workers involved in chemical sterilization using ethylene oxide were examined for elevated SCE in a study by Garry et al.[21] Peripheral lymphocytes from four exposed workers who complained of upper respiratory tract irritation displayed significantly elevated SCE frequencies, as compared to controls, 3 weeks after the last exposure to the gas. The same group of four individuals maintained significantly elevated SCE frequencies (10.3 ±1.8) relative to controls (6.37 ±0.47) as long as 8 weeks postexposure. Eight other

Table 4. Characteristics of the Six Patients Exposed to Arsenic[a]

Patient	Age	Sex	Reason for As admin.	Duration of As Rx (years)	Interval since Rx (years)	Skin Cancer	Comments
1	45	M	asthma	2	26	+	
2	53	M	asthma	0.33	26	+	
3	41	F	psoriasis	2	24	+	
4	70	M	anxiety	0.50	56	+	400 rad to squamous cell carcinoma-1967
5	84	M	asthma	27	1	+	inoperable lung carcinoma; 5000 rad to skin metastasis 1975
6	72	F	asthma	1	33	+	fluoroscopy 2 x weekly while getting As

[a]Taken from Burgdorf et al.[20]

individuals also displayed significantly elevated SCE frequencies between the eighth and ninth week after exposure. Controls consisted of 12 individuals who worked in an adjacent operating room area, who were considered unexposed or incidentally exposed to ethylene oxide. Infrared spectroscopy showed an ambient concentration of 36 ppm ethylene oxide in the hospital sterilization room during the purge/exhaust phase of the sterilization cycle. The open drain from the sterilizer unit showed a concentration in excess of 1500 ppm during the purge phase. The prolonged elevation of SCE in lymphocytes of employees after they had left the working environment was considered unusual by the authors, but this prolonged SCE elevation has been reported for animals studies by Stetka and Wolff.[22]

Melphalan and cyclophosphamide. In another clinical study reported in abstract form, Lambert et al.[33] describe elevated SCE frequency in peripheral lymphocytes from one cancer patient 2 weeks after melphalan treatment (SCE frequency 24.8 ±7.7 SCE/cell per individual, as compared to a frequency of 13.1 ±2.9 for nonsmokers and 16.2 ±3.6 for smokers). Cyclophosphamide-treated patients exhibited SCE frequencies just below the upper limit of control.

Vinyl chloride. Cytogenetic studies were performed on workers at a polyvinyl chloride factory in Norway.[24] Exposure had not exceeded 1 ppm in the past 2 years, and SCE frequencies did not differ from matched controls. For 16 workers, the mean SCE frequency per cell was 7.6, with a range of 4.7 to 10.5. The matched-control mean frequency was 7.5, with a range of 5.2 to 11.6.

In a more recent study concerning 21 workers exposed to vinyl chloride, Anderson et al.[25] found a slight increase in SCE frequency, but this effect was not statistically significant as compared to controls. The 21 workers comprised the second sampling from exposed workers and controls first examined 18 months earlier.[26] Chromosome aberrations in the exposed workers remained significantly elevated, suggesting that chromosomal aberrations may be more sensitive than sister chromatid exchange as an indicator of exposure to vinyl chloride.

Chromosomal Aberration (Bioassay Group 4)

Since aberration studies were frequently carried out in more than one in vitro test system, all such studies are grouped alphabetically by compound. Chromosomal aberration in vivo exposure studies are also organized alphabetically by compound.

Chromosomal aberration in vitro. Arsenic. In a study reported in 1981 by Nakamuro and Sayato[29] of Japan the comparative cytogenetic effects of trivalent and pentivalent arsenic were investigated. The number of chromosome breaks in human leukocytes was significantly higher for the compounds of trivalent arsenic ($NaAsO_2$, $AsCl_3$, and As_2O_3) than those of pentavalent arsenic (Na_2HAsO_4, H_3AsO_4, and As_2O_5). Cultures were treated with arsenicals after 24 h and were harvested after 72 h. This protocol is preferred, since it avoids potential problems associated with blastogenic transformation in the presence of phytohemagglutinin. On an equimolar basis (~2×10^{-6} M), the efficiency in inducing chromosomal aberration was in the order $As_2O_3 > AsCl_3$, $NaAsO_2 >> Na_2HAsO_4 > H_3AsO_4$, As_2O_5. Trivalent arsenic was about 5 times more efficient than pentavalent arsenic in damaging chromosomes. The activity of the compounds with cultured human skin fibroblasts was similar to that in leukocyte cultures. An evaluation of colony-forming capacity after exposure to arsenicals indicated that trivalent arsenic was more toxic than pentavalent arsenic. It was

It was also reported that arsenite (III) was more effective than arsenate (V) in inhibiting incorporation of $[^3H]$-thymidine and ^{14}C-leucine into leukocytes.

Benzene. Koizumi et al.[28] of Japan reported in 1974 that exposure of human leukocytes to benzene in the concentration range of 2.2×10^{-5} to 2.2×10^{-3} M caused dose dependent breaks and gaps. Also, the higher the exposure concentration, the greater the percentage of hypo- and hyperploid cells. It should be noted that benzene did not induce SCE's in human lymphocytes *in vitro* at concentrations up to 1.9×10^{-2} M in one study[14] and 5×10^{-3} M in another.[16]

Chloramphenicol. Goh[29] reported that chloramphenicol, at a concentration of 80 µg/ml (2.4×10^{-2} M), added to lymphocyte cultures at 0, 24, 68, or 71 h produced chromosomal abnormalities (analyzed as vacuoles, dissociations, and translocations) in excess of those observed in controls. No increase in chromosomal breaks was observed. The highest incidence of abnormalities was seen when the compound was added at the G_0 phase of the cell cycle. Results were consistent with the known ability of chloramphenicol to inhibit protein synthesis.

Cyclophosphamide. Several reports were recovered on the cytogenetic evaluation of cyclophosphamide in human peripheral lymphocytes *in vitro*. The first of these by Chebotarev et al.[30] recognized the requirement for metabolic activation and treated cultures of human lymphocytes with the blood serum of mice injected intraperitoneally with aqueous cyclophosphamide solutions of 200 to 1000 mg/kg. Dilutions of active metabolites in the serum were estimated at ten-, fifteen-, or twentyfold in the lymphocyte cultures and were present for 1 h after 32 h of cultivation. Colchicine was added for 2 h prior to fixation at 56 h. A dose dependent increase in chromosome breaks and chromatid exchange figures was observed. An additional report by Madle et al.[31] in 1978 described: 1) the perfusion of rat liver *in situ* with cyclophosphamide and the direct addition of the perfusate to human whole blood cultures and (2) activation of cyclophosphamide with a crude homogenate of mouse liver and its addition to the cultures by means of dialysis bags. The former technique led to dose dependent suppression at mitotic indices and to the induction of chromatid translocations. The latter technique produced similar results when cofactors (NADP and glucose-6-phosphate) were added to the homogenate in a buffered salt solution. A report by Morad and El-Zawahri[32] described an incidence of chromatid breaks of 2, 5.33, and 6% at concentrations of 10, 100, and 1000 µg of cyclophosphamide per milliliter of culture medium, respectively. No exogenous activation was provided for the *in vitro* experiments. Because of the indirect method for applying the test agent reported by Chebotarev et al., and by Madle et al. and the relatively high

concentration of test agent employed by Morad and El-Zawahri, these reports were not considered for comparative analysis.

Diethylstilbestol. An abstract by Bishun et al.[33] indicated that DES was inactive in KB carcinoma cells and human peripheral blood leukocytes at concentrations ranging from 5 to 20 µg/ml (1.9 to 7.4 x 10^{-5} M). No additional details were provided. This report is in contrast with the report by Rudiger et al.[1/] concerning induction of SCE in human skin fibroblasts by DES and two of its metabolites.

Epichlorohydrin. Sram et al.[34] reported that epichlorohydrin induced dose dependent chromosomal abnormalities in human peripheral lymphocytes in vitro at concentrations of 10^{-7} to 10^{-5} M. In that study, lymphocytes were cultured for 56 h, and epichlorohydrin was added 24 h prior to fixation; as a result, 28 aberrant cells were detected among 360 examined at the highest concentration.

Phenytoin. In a 1976 report by Alving et al.,[35] phenytoin was studied in 72 h cultures of human peripheral lymphocytes at concentrations of 10, 50, and 100 mg/ml in the culture medium. The compound was added 4, 24, and 48 h prior to harvest and fixation. In 300 cells counted at each concentration, no significant chromosomal effects were observed, as compared to 300 control cells. These results conflict with the results of Muniz et al.,[36] who reported that phenytoin caused chromosomal damage under identical conditions.

Chromosomal aberration in vivo. Arsenic. A cytogenetic study on arsenic-exposed individuals was reported by Petres et al.[3/] Chromosomal aberrations were observed in peripheral lymphocytes of 31 patients who had a history of arsenic exposure--14 psoriasis patients and 17 vine-growers. All displayed typical arsenic hyperkeratosis on the palms of their hands and the soles of their feet. Several of the patients had had arsenic-induced skin carcinomas excised. The control group consisted of 14 psoriasis patients and 17 healthy volunteers. The frequency of aberrations among the arsenic-exposed subjects was significantly elevated in comparison to controls (Table 5). Of interest was the observed aneuploidy, evidenced as chromosome "deficiency," in the arsenic-exposed population. Although the information contained in this report is incomplete, it provides human data which substantiate the in vitro chromosomal studies[2/] and in vitro[13] and in vivo[20] SCE studies described in previous sections of this report.

Benzene. Though not recovered in the present search, a number of reports have appeared in the literature linking chromosome damage in human lymphocytes to benzene exposure. Pollini and coworkers[38,39,40] published a series of papers describing chromosome damage in men that was related to blood disorders

Table 5. Number of Chromosome Aberrations in 31 Chronic Arsenic Patients and 31 Healthy Subjects[a]

Type of Abnormality	Arsenical Exposure Group	Control Group
Secondary constrictions	52	13
Achromatic lesions	29	3
Gaps	58	9
Chromatid Breaks	34	1
Acentric fragments	39	2
Dicentric chromosomes	3	0
Number of mitoses	1121	1247

[a]Taken from Petres et al.[37]

believed to be caused by benzene exposure. Vigliani and Saita[41] also described the clinical and laboratory details relating benzene exposure and leukemia in six individuals. In 1965, Tough and Court Brown[42] reported that individuals exposed to benzene, but who have no blood disorders, may show chromosome aberrations in cultured peripheral lymphocytes. Forni[43] in 1966 described cytogenic studies of patients who had recovered from benzene-induced blood disorders and of individuals exposed to benzene but who did not show signs of toxicity. In both cases a slight increase in chromosome aberrations was demonstrated. In 1970, Tough et al.[43] published a report on individuals exposed to benzene in three different factories. In two of these groups, the percentage of lymphocytes with unstable chromosome aberrations (e.g., rings, dicentrics, and fragments) was higher than in the general population. However, the observed effects could not be attributed entirely to benzene exposure.

In a study from West Germany by Khan and Khan,[44] cytogenetic evaluation was performed using peripheral blood lymphocytes from 14 controls and 15 workers exposed chronically to benzene. Chromosome studies were performed on bone marrow preparations from four benzene-exposed workers and two controls. For each experimental subject or control, 50 lymphocyte mitoses were analyzed, and a smaller number (number not given) of bone marrow mitoses was scored. The aberrations were classified as chromatid type or chromosome type, and the latter included a) unstable changes, e.g., rings, dicentrics, or fragments, and b) stable monocentric anomalies, e.g., deletions, translocations, and trisomics.

In a more recent study by Picciano[45] 52 workers exposed to benzene at less than 10 ppm were examined for cytogenetic damage in their peripheral lymphocytes. The controls for the study were 44 individuals seen in preemployment examinations, but these control individuals tended to be younger than the exposed workers. Table 6 summarizes the overall cytogenetic data obtained. Picciano scored 200 cells per individual for the presence of chromatid breaks (deletions), chromosome breaks (deletions), marker chromosomes (rings, dicentrics, translocations, and exchange figures), and total abnormal cells. While there appeared to be no difference in the presence of chromatid breaks and total abnormal cells between the two groups, the benzene-exposed workers had twice the percentage of chromosomal breaks and three times the percentage of marker chromosomes.

The workers were divided into two groups according to the length of benzene exposure. Group I included 7 men with 11 to 20 years' exposure; Group II consisted of 8 men with 2 to 5 years' exposure. The percentage of chromatid-type aberrations in lymphocytes was 18.2 in Exposure Group I, 15.0 in Exposure Group II, and 13.2 in controls. Percentages of unstable chromosome anomalies (e.g., rings, dicentrics, and fragments) in Groups I and II (2.3 and 2.2, respectively) were significantly higher than the control percentage (0.3). The percent stable chromosome anomalies (e.g. deletions, translocations, and trisomics) in Groups I and II (2.8 and 1.8, respectively) was also considerably higher than in controls (0.2). The chromosomal analysis of bone marrow cells showed similar changes. The relatively increased rate of

Table 6. Cytogenetic Summary of 44 Nonexposed Individuals and 52 Workers Exposed to Benzene[a]

Cell characteristic	Nonexposed individuals (8800 cells)	Workers (10,400 cells)
Chromatid breaks	1.1%	1.0%
Chromosomal breaks	0.35%	0.67%[b]
Marker chromosomes[c]	0.06%	0.19%[d]
Abnormal cells	1.4%	1.6%

[a] Taken from Picciano.[45]
[b] $t = 2.54$, $P = 0.01$.
[c] Rings, dicentrics, translocations, and exchange figures.
[d] $t = 2.18$, $P = 0.03$.

aberrations in Group I was attributed to a 58-year-old male with benzene myelopathy, who had 30% chromatid-type and 14% chromosome-type anomalies.

Cyclophosphamide. Cytogenetic effects attributed to cyclophosphamide (aneuploidy and structural changes) were reported frequently in the 1960's. Unfortunately, many of these studies were performed in patients with malignant tumors, precluding a determination of whether these aberrations were caused by cyclophosphamide or were a result of neoplasia. Dobos et al.[46] reviewed the earlier studies and demonstrated in nontumorous children undergoing therapy with cyclophosphamide that the agent caused increased breaks (by 18.6%, including chromatid and chromosomal breaks and fragments) after as little as 4 weeks of therapy. This relatively high frequency of breaks was detectable during treatment and for 6 weeks afterwards. After 6 months, chromosomal aberrations returned to almost normal levels.

Epichlorohydrin. Thirty-five workers occupationally exposed to epichlorohydrin were examined in a 1977 study by Kucerova et al.[47] As a control measure, blood samples were collected before the opening of a new plant for production of the chemical. After 2 years of exposure to epichlorohydrin (0.5 to 5 mg/m^3) and of no exposure to radiation or drugs, workers were generally healthy. Four slides were prepared for each blood sample, and, if possible, 50 cells per slide were scored blind by 2 separate laboratories. Chromosomal aberrations were scored as chromatid breaks, chromatid exchanges, chromosomal breaks, and chromosomal exchanges. Gaps were scored separately and were not included in the number of cells with aberrations. Results of cytogenetic analyses from the two laboratories were not significantly different and were pooled. The average percentage of aberrant cells found in blood samples prior to exposure was 1.37; this number increased to 1.91 at the end of the first year of exposure and to 2.69 at the end of the second year. These postexposure numbers were significantly different from the preexposure value, at $P = 0.025$ and $P < 0.0001$, respectively. Chromatid and chromosomal breaks made up most of the increasing number of aberrant cells; chromatid and chromosome exchanges were rare. This study confirmed previous findings with epichlorohydrin and triethylenephosphoramide in human lymphocytes in vitro.[48]

Vinyl Chloride. Purchase and colleagues[49,50] reported in 1975 and 1976 on effects of vinyl chloride. Of the 80 workers studied, 56 had been working in the manufacure of polyvinyl chloride and, therefore, were exposed to vinyl chloride monomer (VCM); the remainder were working in plants and laboratories where exposure to the monomer did not occur. The exposed group consisted of autoclave workers, maintenance workers, and workers involved with the manufacture of vinyl chloride; however, no estimates of exposure levels were provided (but see subsequent report by Purchase et al.[26]).

In Table 7 it can be seen that in the exposed workers, there was a significantly increased ($p < 0.05$) percentage of cells with chromatid or chromosomal breaks (B cells) and of cells with aberrant chromosomes, both stable and unstable. These results confirm previous findings[51,52] and demonstrate that vinyl chloride monomer at sufficent dosage has an effect on chromosomal aberrations in man.

In a study by Leonard et al.,[53] 11 male workers employed in the polymerization department of a vinyl chloride factory, 7 people from a laboratory of another vinyl chloride plant, and 10 controls from outside the factory environment were examined for the presence of chromatid and chromosomal aberrations in peripheral lymphocytes. Although no chromatid aberrations were observed, chromosomal anomalies including fragments, rings, translocations, and dicentrics were seen in lymphocytes from most of the workers from the polymerization department of the vinyl chloride factory. However, it was not possible to determine if the observed chromosomal anomalies resulted from vinyl chloride exposure, since the medical histories of these workers showed that they had received frequent radiographs.

In 1977 Picciano et al.[54] described cytogenetic evaluations of a group of 209 workers employed for up to 28 years in the manufacture of VCM. The findings were compared to results from examination of 295 individuals given preemployment examinations.

Table 7. B Cells[a] and Cells with Chromosomal Aberrations in Vinyl-Chloride-Exposed Workers and in Controls[b]

Workers			Cells with Aberrant Chromosomes	
Category	No.	B cells (%)	Unstable (%)	Stable (%)
Exposed	56	6.30	1.45	0.38
Nonexposed	24	3.63	0.46	0.09

[a]B cells are cells with chromatid or chromosome breaks.
[b]Taken from Purchase et al.[49]

On a group basis, no statistical difference was found between the two groups when they were compared for chromatid aberrations, chromosome aberrations, and proportion of abnormal cells. A comparison of these results with previous reports suggests that the level of cytogenetic aberrations in vinyl chloride workers is probably related to the length and level of exposure. Results are shown in Table 8.

Based upon a 1973-1974 study, a 1978 report by Purchase et al.[26] documented the chromosomal morphology in peripheral lymphocytes of 57 men engaged in manufacturing vinyl chloride or polyvinyl chloride. The control group was composed of 19 on-site individuals and 5 off-site individuals who, for experimental purposes, did not differ significantly. Subjects with recent exposure to x-rays, viral infections, or prolonged drug therapy were excluded from the study. A significant increase in chromosomal abnormalities was observed in peripheral lymphocytes of exposed workers when compared to the combined controls. Greatest statistical significance was observed for the increase in total number of chromatid breaks, chromatid gaps, or chromosome gaps, and in total cells having increased chromosomal abnormalities, both stable and unstable. This increase occurred in autoclave workers who were considered to be exposed to the highest average levels of vinyl chloride. Estimates of exposure (Table 9) were slightly lower than those reported by Hansteen et al. in 1978.[55] (See below.)

Of five other groups having lower average exposure, maintenance workers displayed the greatest increase in total cells with chromosomal abnormalities, as compared to controls; and a miscellaneous worker group (laboratory workers and managers) showed the smallest increase, with only chromatid gaps occurring significantly more frequently than in controls.

Table 8. Cytogenetic Study of Effects of Vinyl Chloride on 209 Workers in Comparison to 295 Controls[a]

Cell Characteristic	Workers (10,483 cells)	Controls (14,761 cells)
Chromatid breaks	2.4%	3.6%
Chromosome breaks	1.0%	1.1%
Rings, dicentrics, and exchanges	0.4%	0.2%
Abnormal cells	3.7%	4.5%

[a]Taken from Picciano et al.[54]

Table 9. Estimated Average Operator Exposure to VCM in PVC Plants[a]

Time Periods	Approximate VCM Concentration (ppm)
1945-1955	1000
1955-1960	400-500
1960-1970	300-400
Mid 1973	15
1975	5

[a]Taken from Purchase et al.[26] Estimates of Barnes.[56]

Workers with a history of exposure to excursion levels of VCM exhibited a higher percentage of abnormal cells. Further analysis indicated that a recent exposure to excursion levels and length of employment were both significantly correlated with chromatid gaps, total C cells, and fragments.

Cytogenetic studies were performed in 1974 by Hansteen and colleagues[55] on 39 workers from a Norwegian polyvinyl chloride plant. Sixteen healthy man not associated with the plant were chosen as controls. Two to two and one-half years later the cytogenetic study was repeated for 37 of the 39 workers. According to the authors, during this time interval, the workers had minimal exposure to VCM. There was a substantial reduction in the estimated exposure to vinyl chloride from 1950 to 1975, as shown in Table 10. This repeated study was performed using 32 matched controls from the office employees in the factory. Forty-eight-hour lymphocyte cultures were prepared, and breaks, gaps, and stable rearrangements were scored in 100 metaphases per person. In the first cytogenetics study, the mean chromosome breakage frequency for the workers (3.41%) was significantly higher than for the controls (1.79%). Bone marrow preparations from four workers were studied in the first investigation. The frequency of mean chromosome breaks was higher (4.2%) in the bone marrows of these four workers than that reported by the authors for normal bone marrows (0.2, 0.4, 1.7%; reference not provided), and higher than for the corresponding lymphocyte cultures. In the repeated lymphocyte study, however, no difference was found in the mean frequency of chromosome breakages between the workers and matched controls. Also, there was no difference between these breakage frequencies and the breakage frequency for the previous control group. The results suggested a relationship between the reduction in exposure to VCM and the normalized chromosome breakage frequency. SCE's were studied for 16 workers and 16 matched controls, and a mean of 7.6 SCE's per cell was found for workers and for controls.

Table 10. Air Concentration of VCM in the PVC Plant[a,b]

Time periods		VCM Concentration (ppm)
1950-1954		2000
1955-1959		1000
1960-1967		500
1968-1972		100
1972-1974		80
1974	Measured	25
1975-		1

[a]Taken from Hansteen et al.[55]

[b]Estimates are based upon the level of production, and the number and types of autoclaves used in the respective time periods.

In a separate study reported in abstract form by Hansteen[5/] in 1978, two young Norwegian workers who had not previously been exposed to any chemical were exposed to 1000 to 3000 ppm VCM for 5 min. Blood samples were taken from the workers and one control individual at 4 h, 28 h, and 7 days after exposure. Lymphocyte cultures were prepared and harvested after 53 h. Breaks, gaps, and rearrangements were scored in 100 cells per person. The total breakage frequency varied in each of the exposed individuals from 0 to 4% for each of the time intervals tested. There was no difference in the frequencies of gaps and rearrangements between the workers and the control. To study SCE's, fresh blood samples and 1-day-old samples stored at 4°C were cultured for 69 and 72 h. The last 48 h of the culture period was in the presence of 3 µg/ml BrdU. SCE frequencies were within the normal range of 7.3 to 10.4 exchanges per cell for both 69 and 72 h cultures of fresh and stored blood for each of the times tested. The authors concluded that high exposure of 1000 to 3000 ppm for the short time of 5 min had no chromosome-damaging effect on blood samples taken 4 h, 28 h, and 7 days after exposure. This study argues against the attribution of any significant chromosomal effects immediately resulting from exposure to vinyl chloride monomer.

The Screening of Human Body Fluids for Mutagenic Activity (Bioassay Group 5)

Among the compounds for which data have been entered into the system, a single abstract describing a study on epichlorohydrin was retrieved. The report by Kilian et al.[58] described direct testing of the compound in S. typhimurium strain TA1535 with positive results. The report indicated that an analysis of the urine of six

workers exposed to 0.8 to 4.0 ppm epichlorohydrin failed to reveal any activity, even in pooled and concentrated samples. However, in the urine of two workers who were inadvertently exposed to a concentration in excess of 25 ppm, mutagenic activity was detected as a conjugate. Mutagenic activity also was reported in the urine of mice after oral administration of epichlorohydrin at 200 to 400 400 mg/kg.

DISCUSSION

The relatively small number of papers recovered in the present data base subset precludes extensive comparison and discussion of findings. However, Tables 11 and 12 reflect our effort to organize information relative to exposure concentration (dose) and response obtained in several genetic bioassays. These bioassays represent four of the five categories of systems mentioned earlier: unscheduled DNA synthesis, DNA strand breaks, inhibition of DNA synthesis, sister chromatid exchange, and chromosomal aberration. The tables include only those compounds for which at least two published reports contained quantitative data for an assay involving human material. Twenty-five publications from the total of thirty-seven contained in the data base subset are represented in the tables. Table 11 provides comparative data for aflatoxin B1, benzidine, cyclophosphamide, and melphalan. Information on diethylstilbestrol, evaluated for unscheduled DNA synthesis, is also shown and may be compared to other data for the same compound shown in Table 12.

It may be seen in Table 11 that Aflatoxin B1 effectively inhibits DNA synthesis (see Footnote d, Table 11) in HeLa cells after a 1 h exposure at only 5×10^{-8} M in the presence of S-9 metabolic activation.[10] Higher concentrations, by three to four orders of magnitude, are required to induce maximal levels of UDS in HeLa cells[4] or in human skin fibroblasts.[6] In the case of benzidine, however, a higher concentration (6×10^{-4} M) was required to effectively inhibit DNA synthesis in HeLa cells in the absence of S-9 metabolic activation[10] than was needed to maximally induce UDS (10^{-6} M) in HeLa cells in the presence of S-9.[4] Similarly, with cyclophosphamide a higher concentration (6×10^{-4} M) was required to effectively inhibit DNA synthesis in HeLa cells[10] or to cause maximal levels of single-strand breaks in DNA (5×10^{-3} M) of human skin fibroblasts[9] than to induce maximum UDS. It should be noted that a longer exposure time, 2.5 h, was employed with the UDS assay[4] than was used with the DNA-synthesis-inhibition assay[10] or the DNA strand breakage assay.[9] Painter[10] used 0.5 h in the absence of S-9 and 1 h in the presence of S-9, whereas Nordenskjold et al.[9] exposed the cells to the chemical for 1 h. In the case of melphalan, the same exposure time (0.5 h) was employed with UDS assays[8] and with DNA strand break assays[9] in human skin fibroblasts. Neither type of assay required S-9

Table 11. Concentrations Required of Selected Compounds to Induce DNA Damage or Inhibition of DNA Synthesis *In Vitro*[a]

Compound	Unscheduled DNA synthesis in HeLa cells (E = 2.5 h)[b]	Unscheduled DNA synthesis in human skin fibroblasts and human bone marrow cells (E = 0.5 h)	Inhibition of DNA synthesis in HeLa cells (E = 0.5 h -S-9, E = 1 h +S-9)	DNA strand breaks in human skin fibroblasts (E = 0.5 h)
Aflatoxin B1	10^{-8} + 10^{-4} M max[c] 10^{-4} M o/+[d] (Martin et al.[4])	10^{-5} + 5×10^{-4} M o/+ (San and Stich[6])	5×10^{-8} M[e] o/+ (Painter[10])	
Benzidine	10^{-7} + 10^{-3} M max[c] 10^{-6} M o/+ (Martin et al.[4])		6×10^{-4} M[e] +/o (Painter[10])	
Cyclophosphamide	10^{-6} M max[c] 10^{-6} M o/+ (Martin et al.[4])		6×10^{-4} M[e] o/+ (Painter[10])	5×10^{-5} + 5×10^{-3} M max[f] 5×10^{-3} M o/+ (Nordenskjold et al.[9])
Diethylstilbestrol	10^{-6} M max[c] 10^{-6} M o/+ (Martin et al.[4])			
Melphalan		10^{-6} + 10^{-3} M max[g] 10^{-3} M +/o (Lewenshon & Ringborg[8])		10^{-5} + 10^{-3} M max[f] 10^{-4} M +/o (Nordenskjold et al.[9])

[a]Appropriate researchers are cited for producing the data retrieved.
[b]E, exposure time.
[c]Maximum dpm/μg DNA above background.
[d]Without S-9/with S-9: o, not tested; +, positive results.
[e]Effective molarity, concentration that inhibited DNA synthesis by 40% within 2.5 h after removal of agent.
[f]Maximum fraction of single-stranded DNA.
[g]Maximum net grain count per nucleus.

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Table 12. Concentrations Required of Selection Compounds to Induce Sister Chromatid Exchange and Chromosome Aberrations in Human Lymphocytes[a]

Compound	Sister chromatid exchange In Vitro	Sister chromatid exchange In Vivo	Chromosome aberration In Vitro	Chromosome aberration
Trivalent arsenic	10^{-6} M & 2 x 10^{-6} M E[b] = 72 h +/o[c] (Zanzoni & Jung[13])	unknown concentration +/o (Burgdorf et al.[20])	6 x 10^{-7} + 7.2 x 10^{-6} M E = 24 h +/o (Nakamura & Sayato[27])	unknown concentration +/o (Petres et al.[37])
Benzene	1.9 x 10^{-5} or 1.9 x 10^{-2} M -/o (Gerner-Smidt & Friedrich[14])		2.2 x 10^{-5} M + 2.2 x 10^{-3} M E = 24 h +/o (Koizumi et al.[28])	< 10 ppm +/o (Picciano[45])
	8 x 10^{-4} + 5 x 10^{-3} M E = 72 h -/o (Morimoto & Wolff[16])			
Diethyl-stilbestrol	8 x 10^{-8} M 2 x 10^{-8} M E = 72 h +/o (Rudiger et al.[17])		1.9 + 7.4 x 10^{-5} M E unknown o/o (Rishun et al.[33])	
Epichloro-hydrin	2 x 10^{-4}, 4 x 10^{-4} M, & 10^{-3} M E = 2 h +/+ (White[18])		10^{-7} M + 10^{-5} M E = 24 h +/o (Sram et al.[34])	

(continued)

Table 12. (Continued)

Compound	Sister chromatid exchange *In Vitro*	Sister chromatid exchange *In Vivo*	Chromosome aberration *In Vitro*	Chromosome aberration
Ethylene oxide	36 ppm E = 24 h +/o (Star[19])	~36 ppm E = unknown +/o (Garry et al.[21])		
Vinyl chloride monomer		<1 ppm -/o (Hansteen[24])		~1-5 ppm -/o (Picciano et al.[54]) ~15 ppm +/o (Purchase et al.[26]) ~25 ppm +/o (Hansteen et al.[55] original study)

[a]Appropriate researchers are cited for producing the data retrieved.
[b]E, exposure time.
[c]Without S-9/with S-9: o, not tested; +, positive results.

activation. A positive response was obtained over a similar concentration range in the two test systems: 10^{-6} to 10^{-3} M with the UDS assay,[8] and 10^{-5} to 10^{-3} M in the DNA strand break assay.[9]

Carefully controlled experiments employing similar conditions of metabolic activation and exposure time in similar experimental systems must be conducted before more definitive comparisons can be made.

In Table 12, comparative data on SCE and chromosomal aberrations may be seen for trivalent arsenic, benzene, diethylstilbestrol, epichlorohydrin, ethylene oxide, and vinyl chloride monomer. For the *in vitro* systems represented in Table 12, the exposure periods are generally longer (24 to 72 h) than with the DNA damage or inhibition assays (0.5 to 2.5 h) discussed in Table 11. Only one compound, diethylstilbestrol, appears in both tables, and, despite the differences in exposure time between the UDS assay (2.5 h) in HeLa cells[4] and the SCE assay (72 h) in human skin fibroblasts,[1/] a positive response was obtained over the same concentration range. The Martin et al.[4] study employed S-9 activation, whereas the Rudiger et al.[9] study did not.

If the results of *in vitro* human lymphocyte assays for SCE and chromosomal abberations in Table 12 are compared, it may be seen that the results of the two assays agreed only for trivalent arsenic in studies by Zanzoni and Jung[13] and by Nakamura and Sayato[2/] and for epichlorohydrin in studies by White[18] and Sram et al.[34] Longer exposure times would appear to account for the fact that lower concentrations of these two chemicals were required to give a positive response in the SCE assays (72 h exposure) compared to the chromosomal aberration assays (24 h exposure). Discrepant results were obtained for *in vitro* SCE and chromosomal aberration assays on benzidine and on DES.

Benzidine was negative in two SCE studies *in vitro* wherein quantitative information was reported[14,16] but was positive for chromosomal aberrations *in vitro*.[28] DES, on the other hand was positive in an *in vitro* SCE assay[1/] but negative for chromosomal aberration *in vitro*.[33]

Human *in vivo* lymphocyte assays for exposure to trivalent arsenic were positive for SCE[20] and for chromosomal aberrations.[3/] No quantitative estimates of arsenic exposure were provided.

In vivo lymphocyte assays on individuals exposed to VCM at low levels were negative (<1 ppm) for SCE[24] and were negative (<1 to >5 ppm) for chromosomal aberrations.[54] Exposure to VCM at higher concentrations (~15 and ~25 ppm) Purchase et al.[26] and Hansteen et al.[55] respectively) resulted in chromosomal aberrations in lymphocytes of workers.

In vitro and in vivo results for a given end point in SCE or chromosomal aberration are in agreement where qualitative information can be compared. Positive responses are observed for induction of SCE in vitro and in vivo with As(III) and ethylene oxide. Chromosomal aberrations are observed in vitro and in vivo with As (III), benzidine, and epichlorohydrin. Quantitative comparisons between the in vitro and in vivo data are exceedingly difficult.

It is apparent that much more work needs to be done in the application of genetic bioassays involving the use of human cells and tissues both in vitro and in vivo. However, it must be emphasized that the chemicals which have been considered in this report and in the previous one (Waters et al., this volume) are among the few of which there is evidence of human carcinogenic potential. For this reason, the completion of the present human genetic bioassay data base is suggested as an appropriately limited research objective for scientists involved in the development and application of such systems. Additional efforts to fill data gaps in the larger data base (Waters et al., this volume) should enhance the utility of the information for purposes of developmental research and quantitative risk assessment.

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